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Temporal Changes in a Natural Population of Copepods

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Abstract. Adaptation was measured in a natural population of the estuarine copepod, *Eurytemora affinis* (Poppe). Significant changes occurred between generations in tolerance to elevated temperature and in body size, in both sexes, and in reproductive traits in females. If these changes were genetic, they did not result in different heritabilities for the traits and genetic variation was maintained in the population. However, genetic correlations between temperature tolerance and brood size in females showed significant changes between generations, lending support to two models for the maintenance of genetic variance. Linkage disequilibrium is discussed as an alternative explanation for the observed genetic changes that took place and for decreased hatchability of broods. Evidence for decreased hatchability, measured as proportion hatch, suggests summer diapause in this species.

Introduction

"How much of the acclimatisation of species to any peculiar climate is due to mere habit, and how much to the natural selection of varieties having different innate constitutions, and how much to both means combined, is an obscure question."—Charles Darwin (1872)

Species can adapt to environmental variation in space or time physiologically, genetically, or by both means (Dobzhansky and Wallace, 1953). Physiological adaptation is accomplished at the individual level independent of available genetic variation. In contrast, genetic adaptation at the population level is dependent on available genetic variation. The degree to which a species uses either or both of these adaptive mechanisms is as obscure today as it was over one hundred years ago.

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Chesapeake Bay populations of the estuarine calanoid copepod *Eurytemora affinis* (Poppe) are exposed to thermal conditions that vary from 0° to 30°C annually. Individuals experience only a portion of this thermal range since their generation time is relatively short (Ketzner and Bradley, 1982). Nevertheless, adaptation to ambient thermal conditions is likely to occur at both the individual and population levels.

A short-term assay of temperature tolerance in *E. affinis* has been used to predict long-term survival in increased temperatures (Bradley, 1976). Sexual dimorphism in this temperature tolerance trait has been observed repeatedly (Bradley, 1978a, b, 1982). Males expressed more additive genetic variance and occasionally less physiological tolerance for elevated temperatures than did females. However, physiological flexibility, measured as the difference between tolerances before and after acclimation to temperatures higher than those of standard culture regimes, was greater in females.

Genetic adaptation seems to be relatively less important to this species since individuals can survive and reproduce throughout the entire range of thermal conditions to which they are exposed. In spite of its physiological range, *E. affinis* did respond genetically with a sufficient rate of thermal change indicating that variation is both present and usable (Ketzner and Bradley, 1982). The question to resolve is why this species maintains genetic variation (Slobodkin and Rapoport, 1974).

There is no evidence for either dominance genetic variance or genotype by environment interaction in temperature tolerance in *Eurytemora* (Bradley, 1978a, b, 1986). Thus, selection models based on either temporal or spatial environmental variation (Gillespie, 1974; Haldane and Jayakar, 1963; Levene, 1953), or on a combination of the two (Ewing, 1979), are unlikely to account for the variation (Bradley, 1982). Similarly, heterozygote superiority appears unlikely since dominance is neces-

sary for this phenomenon. Other models are more difficult to disprove. Density-dependent selection is probably absent since it would require a form of heterosis to maintain genetic variation. Frequency-dependent selection cannot be seriously considered without examining linkage relationships among many individual genes. Finally, stabilizing selection, even without heterosis, cannot be ruled out if mutation rates are high (Lande, 1975), which seems unlikely in this species.

Cyclical (seasonal) selection seems an obvious explanation for maintenance of genetic variation (Bradley, 1982, 1986). However, there was no consistent pattern among seasons for temperature tolerance among progeny taken from wild females and raised at 15°C in the laboratory. Neither was there a consistent pattern for seasonal progeny tested contemporanously; parents from different seasons were maintained at 4°C (at which temperature generation time is about 4 months) until all collections were made and their progeny isolated and reared simultaneously at 15°C prior to testing (Bradley, unpub.). There is actually some indirect evidence against cyclical selection. Tolerances to high and low temperatures appear to be the same trait within both sexes, or at least are not negatively related (Bradley, 1976, 1986). Second, genotype by environment interaction for temperature tolerance, another potential indicator of seasonal selection, seems to be absent (Bradley, 1982, 1986).

Two models of selection to account for the maintenance of genetic variation in these copepods are still under serious consideration. One model is disruptive selection between the sexes (Mather, 1955), in which *E. affinis* females are selected for physiological flexibility and males for absolute temperature tolerance. Some evidence exists for the required genetic variation for flexibility (Ketzner, 1979). There is also some evidence for a negative correlation between absolute temperature tolerance and flexibility, and for differential selection between the sexes (Bradley, 1982). Under this model, genetic variation for both traits is preserved if selection is for a different trait in each sex.

An alternative model, genetic homeostasis (Lerner, 1954), states that genetic variation is maintained because selection for temperature tolerance is resisted by natural selection for fitness. This model requires genetic variation for temperature tolerance and for some other component of fitness, and a negative genetic correlation between the two traits. Temperature affects egg production, growth rate, size, and longevity in *E. affinis* (Heinle, 1970; Katona, 1971) and such traits may well undergo natural selection, allowing the possibility of genetic homeostasis. There is also some evidence for seasonal divergence in viability in *Eurytemora* (Bradley, 1982).

The issue in both the disruptive selection and homeostasis models is the sign and magnitude of the genetic correlations. The present study was designed to determine genetic correlations between temperature tolerance and other traits influenced by temperature or related to fitness of *E. affinis*.

Materials and Methods

The calanoid copepod *Eurytemora affinis* (Poppe) (Arthropoda, Crustacea) has peak densities during late winter and early spring in Chesapeake Bay beginning when water temperatures approach 5°C. It can be found throughout the year and is, therefore, exposed to a wide range of ambient temperatures (approximately 0–30°C). The actively swimming population is greatly reduced in summer and early fall.

Two samples (March and May, 1984) of *E. affinis* representing populations at early and late peak densities were taken from Bear Creek (39° 15′ latitude, 79° 27′ longitude, approximately). Temperatures *in situ* were 5.0 and 18.0°C, respectively. Ambient temperatures measured during previous years during the same periods also differed by more than 10°C. Based on generation times at various temperatures (see Katona, 1971), we assumed that adults present in the samples represented two distinct generations. We believe that predation by fish larvae reduced any generational overlap.

Ovigerous females were isolated into 50 ml of filtered bay water in 125-ml Erlenmeyer flasks within a few days of sampling. Each parent was removed when its egg sac was shed. Therefore the progeny in each flask were sibs. A 4:1 mixture of two algal species—*Monochrysis lutheri* (supplied by Howard Seliger's lab at Johns Hopkins University) and *Nitzchia* sp. (isolated from Bear Creek)—in log phase growth was added, as necessary, to maintain food concentrations near 10^5 cells per ml. Each parent was removed when its egg sac was shed. Progeny were raised in the same flasks under standard growth conditions (15°C, 14/10 L/D cycle, moderate light intensity) until ovigerous females appeared, whereupon members of both sexes from each sib group were isolated for testing.

All water used in the study came from Bear Creek and was stored at 4°C in 20-l polypropylene carboys prior to use. The treatment of bay water for algal food stocks differed from that used for copepod cultures in that algal water was charcoal-stripped to remove organic material (2 g powdered, HCl-washed, activated charcoal per liter stirred for 1 h), filtered through a .45-μm Millipore filter (HAWP 047-000) preceded by a Millipore cellulose prefilter (AP25-035-00) and autoclaved for 20 min at 121°C, 15 psi. The finished product stood at least 24 h to permit equilibration with atmospheric gases prior to use. Water for copepod culture was only filtered, as above, with the exception of water used for preliminary and reproduc-

tion studies. Early studies indicated no significant differences between the two water types when used for copepod cultures.

Filtered bay water was dispensed in 500-ml portions into 1-l Erlenmeyer flasks with 38 mm Ka-put tops (for algal cultures) or 1-l portions into 2-l flasks with screw caps (for general use). Plastic, disposable petri dishes were used as covers on the flasks to minimize evaporation yet allow sufficient air circulation. Sterile disposable 60 × 15 mm petri dishes (Falcon No. 1007) with 5-ml portions of treated bay water plus 5 ml food were used in many preliminary and reproduction studies.

Nutrient enrichments were added to the algal cultures. ESI_{Si} enrichment (Provasoli, 1968) was added at 2%. A second enrichment, a modification of a B-vitamin mix formulated by Lewis (1967), was filter-sterilized and added at 0.5%. Adsorption of the vitamins in the B-vitamins mix onto algae promoted the growth and fertility of copepods better than direct addition of the vitamins to copepod cultures. Detailed culture methods are provided in Tepper (1986).

Parental and F_1 brood sizes were measured by isolating ovigerous females on petri dishes and counting under a dissecting microscope (25× magnification) only eggs within the brood sac. Ovigerous females were isolated in petri dishes with water and food (as above) or in vials. In the former case, proportion hatch (PH) of first brood was calculated as the number of hatched eggs divided by the brood size. Proportion hatch data were arcsin transformed prior to analysis. Survival after hatching of individuals in laboratory culture was near 100% and 70–80% of parental females released viable broods.

Heat tolerance (HT) was measured with a short-term nondestructive assay which correlates well with survival in elevated temperatures (Bradley, 1976). A modified version of the assay was used in this study consisting of immersing animals contained in individual vials into a computer-controlled water bath initially set at 32°C and raising the temperature 0.5°C every 5 min. HT was measured as the time taken for animals to succumb, that is to fail to respond to light shaking of the vials. Vials were prepared from Pyrex tubing, 21 mm ID by 100 mm high, which was fire polished and fitted with Nytex mesh (74 μm) on the bottom end. A run (one assay) consisted of a group of 16 vials, held 2 deep in a plexiglass rack, immersed simultaneously into the test tank (standard 10 gal aquarium) containing 5 ppt Instant Ocean made with distilled water. Each run contained four sib groups of four copepods each. Generally only families with at least four individuals of at least one sex were included in the analysis. A few broods with only three individuals of a sex were included. Sexes were tested separately. Thus the average tested brood size was around eight.

Protosomal (body) width (BW) was measured after the tolerance assays using a Zeiss compound microscope fitted with an ocular micrometer under 100× magnification. All measurements were taken on animals arranged dorsal-side up on depression slides. When immediate measurement was not possible, animals were stored at 4°C to arrest growth.

Seasonal genetic adaptation was suspected in previous studies (Bradley, 1982) and preliminary analysis indicated a difference between March and May samples in 1984. To further explore the possibility of temporal genetic adaptation, the principal comparisons made were between samples within sex. Statistical analyses included: means of sample family means and their variances, full-sib heritabilities, and genetic correlations based on family means. Student *t*-tests were used for within sex, between sample phenotypic comparisons of family means for all traits.

Full-sib estimates of heritability (h^2) were derived by partitioning the among-group mean square of a nested analysis of variance of families within tolerance runs into variance components (Falconer, 1981; Robertson, 1959a). Nesting was necessary to remove variation due to the physiological (HT) assay. An unbiased estimator of "average" or adjusted family size was used to calculate the maternal variance component (Brownlee, 1965). The exact variance of an h^2 estimate was derived from the variance of the intraclass correlation coefficient (t) among families within runs between phenotype and genotype (Fisher, 1941).

Genetic correlations (r_A's) between temperature tolerance and other traits were calculated using family means for each trait. Means tend to be normally distributed irrespective of the underlying distribution of the individual-within-family data (Kleinbaum and Kupper, 1978). Genetic correlations were calculated from a nested analysis of covariance based on family means. The covariance between a pair of traits was divided by the square root of the product of variances for each of the two traits. These estimates define the extreme limits of the genetic correlations in the same way that heritability estimates from full-sib analysis are upper estimates (Mode and Robinson, 1959). The standard error of each genetic correlation was calculated according to Robertson (1959b).

Two sets of data for female reproductive traits were available. First brood size was measured on the same individuals used for width and temperature tolerance measurements. A separate set of first brood sizes, as well as the proportional hatch of these broods, was measured on a second set of female sibs from the same families. The measurements of brood size were distinguished from one another as "MB1" and "RB1," respectively.

Results

The results of F_1 phenotypic comparisons between the two samples for the various traits are summarized in Ta-

Table I

Comparisons of first filial phenotypic means (of family means) and full-sib heritabilities from March and May 1984 sampling periods

Sex	Trait	Sample	Inds.[a]	Fams.[b]	Mean	S.D.	t	h^2	S.D.	df[c]	t
M	High temp. tol.	March	154	41	12.26[d]	2.35	**	.32	.19	30	n.s.
		May	187	50	8.53[d]	2.81		.72	.19	36	
F	High temp. tol.	March	163	41	13.82[d]	3.35	**	.90	.19	30	n.s.
		May	208	53	7.85[d]	2.33		.74	.18	38	
M	Width	March	155	41	326.04[e]	18.24	**	1.18	.17	30	n.s.
		May	183	50	309.78[e]	14.74		1.09	.17	36	
F	Width	March	162	41	443.84[e]	23.41	**	1.22	.17	30	n.s.
		May	209	53	421.65[e]	29.49		.99	.17	38	
F	M-brood size	March	164	41	44.14[f]	12.83	**	.91	.19	30	n.s.
		May	212	53	31.58[f]	12.02		1.16	.15	38	
F	R-brood size[g]	March	164	41	36.76[f]	11.73	**	1.07	.18	30	n.s.
		May	209	53	30.00[f]	11.57		1.41[i]	.12	38	
F	Proportion hatch[g]	March	164	41	.35[h]	.26	**	1.30	.15	30	n.s.
		May	209	53	.05[h]	.14		1.48[i]	.11	38	

[a] Individuals among families tested, [b]number of families tested, [c]number of families less number of tolerance runs (within sample), [d]minutes (time to succumb), [e]micrometers (converted from ocular units), [f]egg number, [g]represents a separate set of sibs from the same families, [h]proportion of hatched eggs (arcsin transformed prior to analysis), [i]significantly greater than 1.00 at $P < .05$, ** = $P < .01$, n.s. = $P > .05$.

ble 1. Temperature tolerance and size decreased significantly ($P < .01$) in both sexes between March and May samples. Length was also measured, but is not reported because no differences between length and width were found. Both measures of brood size and of proportion hatch decreased significantly ($P < .01$) in females during the same period.

In general, temperature tolerance increased proportionally with increasing ambient temperature due, in part, to acclimation (Bradley, 1978a). The temperature tolerance of wild-caught females, many of whom produced the progeny used in full-sib analyses, increased between the sample periods ($t = 2.63$, $P < .01$, $n_1 = 39$, $n_2 = 94$). Consequently, the decrease in temperature tolerance among F_1s between samples was puzzling initially. Physiological adaptation to laboratory thermal conditions (15°C) may have resulted in long-term acclimation by progeny from March in an upward direction and from May in the opposite direction. The opposing directions of acclimation by progeny would account for the discrepancy in these results. Parents had been in the laboratory two days before they were tested. Consequently, the results for wild-caught females represent conservative estimates of increased HT, relatively unbiased by laboratory acclimation, since complete acclimation requires additional time (Bradley, 1978a).

Decreased F_1 brood size between samples is consistent with previous results (Bradley, 1982; Katona, 1971). Decreased proportion hatch (PH) between samples is consistent with a previously found decrease of egg-to-adult viability for individuals grown under various temperature regimes in laboratory cultures (Bradley, 1982). Some of this variation may be maternal.

Estimates of F_1 heritabilities are also summarized in Table 1. Neither sex showed significant changes in heritability estimates between March and May samples for any of the traits studied. The estimates were surprisingly high.

The maximum theoretical value for heritability is 1. Many of the estimates exceed this and, for two of the traits, RB1 brood size (second set) and proportion hatch in the May sample, the 95% confidence intervals do not include the maximum theoretical value. Possible causes of biases in heritability estimates are discussed later.

The results of pairwise F_1 genetic correlations (r_A) calculated between temperature tolerance and the other traits are summarized in Table II. The genetic correlations were calculated from family means for each trait.

Table II

Comparisons of first filial genetic correlations (based on family means) between temperature tolerance and other traits from March and May 1984 sampling periods

Sex	Trait	Sample	r_A	S.D.	N[a]	df[b]	Z[c]
M	Width	March	.04	.21	41	27	n.s.
		May	.06	.14	50	33	
F	Width	March	−.16	.12	41	27	n.s.
		May	−.29[d]	.13	53	35	
F	M-brood size	March	.18	.14	41	27	*
		May	−.35[d]	.11	53	35	
F	R-brood size[e]	March	.35[d]	.12	41	27	**
		May	−.37[d]	.09	53	35	
F	Hatch percent[e]	March	.02	.11	41	27	n.s.
		May	−.31[d]	.09	53	35	

[a] Number of families tested, [b]number of families minus runs minus 3 for [c]Z test scores (Kleinbaum and Kupper, 1978), [d]significantly different from 0 at $P < .05$, [e]represents a separate set of sibs from the same families used for other traits, * = $P < .05$, ** = $P < .01$, n.s. = $P > .05$.

They provide estimates of the limits of genic correlations (the contribution of additive genetic covariance to the phenotypic covariance) since some of them may be increased by dominance and common environmental effects. Five of the eight genetic correlations from females were significantly different from 0 ($P < .05$), suggesting either pleiotropy or linkage disequilibrium (nonrandom associations between the genes for the different traits, perhaps due to their being on the same chromosome).

No change in the genetic correlation between HT and width was evident in either sex. However, females did show a significant change, from positive to negative, in r_A estimates between HT and brood size (with MB1 $P < .05$, with RB1 $P < .01$). The r_A between HT and proportion hatch also changed, from positive to negative, but not significantly.

Discussion

Temporal changes in temperature tolerance

Individuals sampled from March and May were exposed to quite different environments in the field during their development from egg to adult. The March generation experienced thermal conditions that cycled from 5° to near 0° and back to 5°C. In contrast, the May generation was exposed to increasing temperatures (from 5° to 18°C). The phenotypic differences observed in family means of laboratory-reared progeny suggest genetic changes occurred in the natural population, probably in response to parental exposure to field variation in thermal conditions. However, adaptation was not accompanied by a reduction in heritability (h^2) in either sex.

Heritability estimates for HT from male progeny were similar to previous findings (Bradley, 1978a, b, 1982). In general, female estimates were four times as large as previously found. No previous estimates of heritability for any of the other traits studied were available for direct comparison. Estimates of h^2 are often biased upwards when full-sib analyses are used due to (a) dominance (V_D) or common environment (V_{Ec}) components of variance and (b) large standard errors due, in part, to environmental differences under which selection (*in situ*) and testing (*in vitro*) take place (Bohren, 1975). However, in previous studies on the temperature tolerance trait either dominance (Bradley, 1978b) or maternal effects (Bradley, 1978a) were found.

Rearing conditions can produce a dominance-like effect (Istock, 1981) by presenting genotypes with novel environments for which prior genetic adaptation has not occurred. Laboratory procedures for the present study differed from earlier experiments in food type, quality, and concentration. Consequently, female heritabilities that were higher than previous estimates, might be inflated by V_D. Estimates of h^2 have varied with thermal or salinity rearing conditions, although not significantly (Bradley, 1986). Similar nonsignificant differences among heritability estimates due to small differences in thermal rearing conditions (10°, 12.5°, and 15°C) were found for *Eurytemora herdmani* (McLaren, 1976). In the present study, males should have had similarly inflated heritabilities over previous estimates, since temperature tolerance is apparently the same trait in both sexes (Bradley, 1978a); however, male estimates were somewhat lower than expected.

Previous results suggested that seasonal genetic diversity for HT in *E. affinis* was small or nonexistent (Bradley, 1982). The conclusion was supported by the observation that both heat and cold tolerance were either influenced by the same set of genes (*i.e.*, tolerance to both thermal extremes is the same trait) or at least were not negatively related (Bradley, 1976, 1978a, 1986). The same appears to be true of a desert pupfish; those from more thermally variable habitats were more resistant to both high and low temperature stress than pupfish from thermally stable habitats (Hirshfield, *et al.*, 1980). Other supporting evidence comes from the lack of genotype by environment interaction, which would be expected with seasonal diversity (Bradley, 1982, 1986).

The absence of genetic response in HT between samples is also consistent with a laboratory selection study

by Ketzner and Bradley (1982) in mass cultures of *E. affinis*. In the present study the rate of *in situ* thermal change represented an increase of 0.21° per day (1.49° per week). Response to a variable *in vitro* temperature regime was primarily genetic when the rate of cyclical temperature change (between 10° and 22°C) was rapid (1°C per day), but was primarily physiological when the temperature changed 1° per week.

Temporal changes in other traits

Mean body widths in each sex, brood sizes, and proportion hatch all decreased significantly from March to May, but heritabilities generally remained constant and high.

There was a decrease in mean proportion hatch (PH), although once again heritabilities remained constant between samples. The decrease in PH may represent summer diapause in this species. Johnson (1980) has reported resting eggs in *Eurytemora*. Evidence of diapause in this study comes from the following three observations of the data. First, proportion hatch data are surprisingly consistent within families. All females within a family tended to have either greater than .80 or less than .20 proportion hatch. Families with a mix of high and low PHs were rare (<5% of population), as were females with intermediate PH values. Second, population mean PHs significantly decreased from March to May, and from the previous December to March (Tepper, 1986), suggesting a systematic decline in PH culminating in summer reductions in *E. affinis* populations. It remains to be shown that shed unhatched eggs are viable and that some environmental cue stimulates their continued development. Third, h^2 estimates for PH, which were very high, showed no change between samples. If summer diapause does occur, then the response would be purely physiological within a given habitat. Experimental selection for a change in the trait based on an environmental cue would further substantiate the relationship between diapause and proportion hatch.

The temporal genetic changes reflect natural phenomena occurring during the span of one or possibly two generations. There is ample precedent for rapid response to selection from artificial breeding experiments (see reviews by Falconer, 1981; Wright, 1977) and from natural populations (see review by Wright, 1978). Hairston and Walton (1986) recently inferred natural selection due to predation in freshwater copepod populations. They observed a rapid directional shift in timing of diapause when fish predators were no longer present. Seasonal and annual differences due to natural selection have been recorded for copepods (Battaglia and Lazzaretto, 1967) and bird populations (Smith and Zach, 1979; Boag and Grant, 1981). The pitcher plant mosquito produces several generations a year and apparently undergoes continuous natural selection for shifting degrees of larval diapause (Istock, 1981).

Laboratory results are often viewed as unrepresentative of values in nature since heritabilities and genetic correlations are functions of the environment in which they are measured (Stearns, 1976; Via, 1984). The absolute magnitude of the estimated genetic parameters is less important than change from sample to sample. Genotype by environment (G × E) interactions affect laboratory estimates of genetic parameters proportional to the degree *in vitro* conditions fail to meet the biological requirements of the organism (Robertson, 1957). The laboratory conditions used may have been stressful to *E. affinis*, but they were stressful to both samples. In addition, the relative contribution to phenotypic variance of G × E interactions is generally small (Falconer, 1981) and several studies have failed to find G × E interactions in natural populations (*e.g.*, Boag and Grant, 1978; Bradley, 1982, 1986; Boag, 1983; Murphy *et al.*, 1983).

Interactions among traits

Given sufficient physiological flexibility to meet most or all environmental circumstances, genetic adaptation would seem unnecessary. An early prediction was that genetic adaptation would be less important in females than males since female *E. affinis* have greater physiological flexibility and less additive variance for temperature tolerance than do males (Bradley, 1978a, b, 1982). However, genic (additive) variation was maintained for temperature tolerance in *E. affinis*, suggesting either varying selection pressure on males or periodic resistance to selection for temperature tolerance due to negative relationships with other traits that are also under selection pressure. The estimates of heritability of progeny reared in the laboratory were similar between samples. However, genetic changes from March to May are suggested by the changes in means of laboratory-reared progeny in all the variables.

The genic relationship between temperature tolerance and reproductive traits varied between samples suggesting differential selection pressures among traits in *E. affinis*. The genetic correlations between temperature tolerance and brood sizes, and between temperature tolerance and hatch percent consistently changed sign from positive to negative (Table II). This is the expected pattern. If selection were for both traits, pleiotropic genes affecting both traits in the same direction relative to selection would be fixed. Thus only pleiotropic genes affecting traits in opposite directions would contribute to the genetic correlation, tending to make it negative.

It is also possible that female *E. affinis* are selected for intermediate levels of egg production and temperature

tolerance, due to competing energy requirements. In males such compromise may be unnecessary if sperm production requires less energy.

Trade-offs are common among life history characters in females of other species (Primack, 1978; Rose and Charlesworth, 1981a; review: Stearns, 1976) and directional selection applied to one life-history trait often results in a correlated response in another, or in several others. Selection for decreased development time resulted in a decrease in percent diapause in *Wyeomyia smithii* (Istock *et al.*, 1976). Selection for early fecundity resulted in increased early egg production whereas selection for late fecundity resulted in the opposite and in increased longevity in *D. melanogaster* (Rose and Charlesworth, 1981b; Luckinbill *et al.*, 1984). In the same species, selection for postponed reproduction increased longevity and decreased mortality rates (Rose, 1982). Laboratory adaptation in mass cultures of *Gammarus lawrencianus* for increased survival, early maturity, and high fecundity produced increased life expectancy and growth rate (Doyle and Hunte, 1981).

There are also cases in which balances among traits were absent: *e.g., Gambusia affinis* (Trendall, 1982), *D. simulans* (Murphy *et al.*, 1983), and *Thamnophis elegans* (Arnold, 1971). These exceptions may represent an inappropriate choice of traits for a particular species or inadequate sample sizes. They may also reflect more versatile homeostatic mechanisms.

Linkage disequilibrium or pleiotropic response?

Genetic correlations persist if caused by pleiotropy, but not if they result from linkage (Hazel, 1943; Lande, 1980). Since the observed changes were short term, they may have been due either to extensive linkage caused by unusual meiosis or to pleiotropy.

Linkage, in general, is unimportant for complex traits since these traits tend to be controlled by many genes scattered over several chromosomes (Lande, 1979; Stearns, 1980; Wright, 1977). However, work on *Drosophila* heat-shock genes indicates linkage among the genes involved in synthesis of the smaller of such proteins. Moreover, heat resistance in *Drosophila* can be modified through selection (Morrison and Milkman, 1978; Pelham, 1982). *E. affinis* has a set of heat resistance genes, some common to *Drosophila* and other organisms, and some not (Bradley *et al.*, 1988). The linkage relationships among these genes in *Eurytemora* is not known. *Drosophila* has many fewer linkage groups than does *Eurytemora*, so we cannot immediately argue the importance of linkage.

The absence of crossing over during gametogenesis (achiasmata) can cause linkage disequilibrium (non-random associations of genes) since gene combinations present in the parent are transferred intact to its offspring. Achiasmate females are common among invertebrates and can include entire orders (*e.g.*, Lepidoptera) or suborders (*e.g.*, the dipteran Brachysera) or only individual species within higher taxonomic classifications (White, 1973). Both cyclopoid and harpacticoid copepods are achiasmate in females and seem to be chiasmate in males (Ar-Rushdi, 1963; Wyngaard and Chinnappa, 1982). Thus, the consequences of linkage may be present in copepods, even with many chromosomes.

The occurrence of chromosomal ring structures during meiosis results in non-random segregation of chromosomes (White, 1973) and, therefore, can also produce linkage disequilibrium involving much of the genome during oogenesis. Cytological studies are rare for calanoid copepods, but the related freshwater cyclopoid species, *Mesocyclops edax*, has ring structures among chromosomes (similar to those formed in *Oenothera*). These are frequently formed during female meiosis (Chinnappa and Victor, 1979). Similar rings are formed during female gametogenesis in three species of harpacticoid copepods belonging to the genus *Tigriopus* (Ar-Rushdi, 1963). In *M. edax*, the number (one or more) and types (number and kind of chromosomes included) of rings can vary within and between populations, although within individuals the number and types were constant (Wyngaard and Chinnappa, 1982). In some cases, all *M. edax* chromosomes ($2n = 14$) were involved in one ring, creating two linkage groups during meiosis; one for each sex in the offspring, females being the heterogametic sex.

Whether in *E. affinis* ring formations occur during oogenesis is not known. Nor is it known if females are achiasmatic and heterogametic. It is clear that if *E. affinis* is similar to *M. edax* and *Tigriopus* species, which is likely, then the observed results could easily be the product of disequilibrium. The number of chromosomes involved in ring formation may be related to ambient thermal conditions or some other environmental cue. Further research in the cytology of *E. affinis* is warranted.

Ring formation in *E. affinis* could help explain the observed decreases in proportion hatch measured in progeny. Recessive lethals in female parents linked in ring structures could be passed on to progeny intact. If females are the heterogametic sex, then female progeny could be lost due to sex-linked lethals. McLaren and Corkett (1978) reported an unusual shift in the sex ratio of a population of the copepod *Pseudocalanus*. If ring structures occur in both sexes during gametogenesis, then inviable offspring would be even more prevalent.

The alternative explanation invoking pleiotropy can be offered for the changes observed in genetic correlations. As we suggested earlier, a trade-off, influenced by thermal variation in ambient conditions, may exist between temperature tolerance and brood size in fe-

males. Under this hypothesis, seasons represent different micro-environments to which the sexes adapt independently, not unlike micro-adaptation by individuals within seasons (Van Valen, 1965). Cyclic seasonal selection should then reverse the observed genetic correlations between HT and brood size in females during fall and winter.

Both linkage and pleiotropy may be important in the relationships between temperature tolerance and the other traits. Strong directional selection in large populations can fix either pleiotropic or linkage groups and allow those genes with antagonistic effects to segregate (Lande, 1982). The change in genetic correlations between temperature tolerance and brood size from positive to negative may have been the product of antagonistic genes. Although most of the changes between sampling periods were physiological, the thermal difference between samples was sufficient to produce some genetic adaptation. Earlier studies with *Eurytemora* found that beyond certain rates and ranges of change in temperature, genetic adaptation occurred. Exposure of copepods to differential temperatures in cooling waters through a power plant was sufficient to elicit a genetic response in HT of males (LaBelle and Bradley, 1982). Genetic adaptation also occurred during *in vitro* selection with the same magnitude of temperature change (Ketzner and Bradley, 1982).

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